

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: Hagio *et al.*

SERIAL NO.: 10/521,622

FILED: January 14, 2005

FOR: ELECTROPORATION METHOD
INCLUDING THE USE OF
DEPRESSURIZATION/PRESSURIZATION

EXAMINER: Fernandez, S.E.

ART UNIT: 1651

CONFIRMATION No.: 2017

Declaration Under 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
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Alexandria, VA 22313-1450

Sir:

I, Dr. Takeshi Hagio, hereby declare that:

1. I have been employed as a staff scientist at the National Institute of Agrobiological Sciences, Ibaraki, Japan, the assignee of the above-identified application, since 1992.
2. I hold a Ph.D. in agriculture from Okayama University, Japan, in 1998.
3. I am one of the inventors in the above-identified application and am fully familiar with the subject matter thereof.
4. I was directly involved in the studies described below to test the activity of antisense conjugates of various arginine-rich peptides both *in vitro* and *in vivo*.

Materials and Methods

5. Two different strains of rice were tested for transfection efficiency: KOSHI-HIKARI and KITA-AKE. The seeds were prepared for transfection as followed. The seeds from each strain were allowed to absorb water at 25°C overnight. Into a petri dish, the following were placed a) 2 mL of an electroporation buffer solution, b) 30 seeds which begun spouting, and c) plasmid DNA (200 µg/ 2mL) of pWI-GUS containing GUS or pWI-H5K containing npt II. Depressurization was then performed for one hour at a pressure corresponding to the atmospheric pressure reduced by 0.096 Mpa. Thereafter, the seeds and the buffer solution were transferred from the Petri dish to a chamber. The chamber was placed on ice for one minute.

6. Transfection of the rice seeds was accomplished as follows. An electric pulse was applied under the following conditions: a) the voltage was 50 V/cm, b) the pulse width was 50 milliseconds, and c) the number of pulses was 50. In experiments, the electrode potential was not changed. We used the parallel electrode chamber (figure.1).

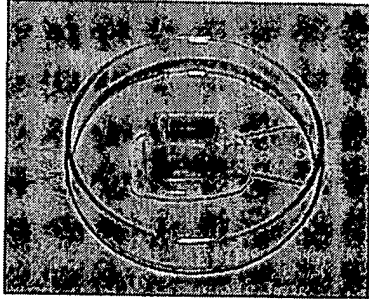


Figure 1. Parallel electrode chamber

7. In order to evaluate the efficiency of transfecting pWI-GUS, a plasmid containing GUS into rice was measured. Rice that express a high level of GUS, as shown by the symbol "+", are scored as having 10 points. A moderate level of GUS expression, as shown by the symbol "±", are scored as having 5 points. Rice that do not express GUS, as shown by the symbol "-", are scored as having zero points. Exemplary scored rice are shown in Figure 2.

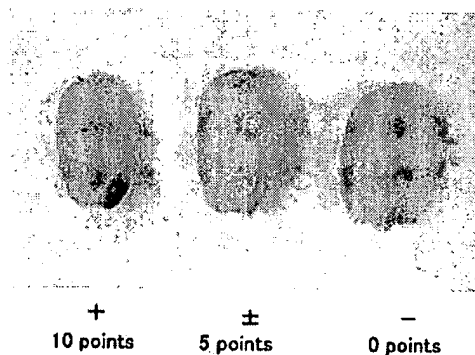


Figure 2. Exemplary KOSHI-HIKARI rice scored for pWI-GUS transfection efficiency.

Results

8. Twenty of rice from each strain, KITA-AKE and KOSHI-HIKARI, were tested for transfection efficiency. The mean GUS expression scores for KITA-AKE and KOSHI-HIKARI were 2.5 and 2.0 respectively. The above data confirms the efficient transfection is achieved by the above procedure.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of any patent issuing from this patent application.

Date: April 3, 2008

Takeshi Hagio

Dr. Takeshi Hagio